PACEMAKER PROPERTIES OF TUNICATE HEART CELLS

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The primary pacemaker regions are located at the cardiovascular junctions and they alternate in periods of activity which change the direction of peristalsis (see Kriebel, 1968a). It will be demonstrated below that isolated sectors of a cardiovascular junction show about equal pacemaker capabilities and when more than one region is independently active, arrhythmia results. The region which usually functions as the pacemaker was determined and its interaction with latent pacemaker regions is discussed below.

The myocardium is composed of a single layer of musculoendothelial cells, except for a line of undifferentiated cells located opposite the cardio-pericardial raphe; these undifferentiated cells resemble those forming the ring of undifferentiated cells at the ends of the heart (see Millar, 1953). Many investigators have demonstrated that narrow rings of tunicate hearts can beat rhythmically (Bancroft and Esterly, 1903; see Krijgsman, 1956, for a review). However, rings of the heart contain both the raphe and the undifferentiated line of cells, which raises the question whether the myocardial cells themselves have pacemaker properties or whether pacemaker activity is limited to the undifferentiated cells. To investigate this question pacemaker capabilities of pieces of myocardium were examined after both the undifferentiated line and the raphe had been removed.

Methods

Hearts of adult *Ciona intestinalis* (from Woods Hole, Massachusetts and from California) were used for most experiments. For comparative purposes, hearts of adult *Chelysoma productum*, *Ascidia callosa* and *Corella willmariana* (from Friday Harbor, Washington) were also used.

Heart contractions in intact animals were observed with the aid of a dissecting microscope and recorded on a kymograph drum. Dissections were performed in sea water at 10° C. *Ciona* heart action potentials were recorded *in situ* by applying suction electrodes to the raphe so that portions of both pericardium and myocardium were sucked into the electrode openings. Electrical activity of the pacemaker regions was recorded by placing as many as four electrodes on the myocardium near the cardiovascular junction. In a second method of recording action potentials, hearts were opened and positioned over a Plexiglass plate containing a row of small electrode openings spaced 1-mm. apart (see Kriebel, 1967a, for details).

Small pieces of heart were spread onto a microscope slide moistened with sea water between two ridges of petroleum jelly. A coverslip holding a drop of sea

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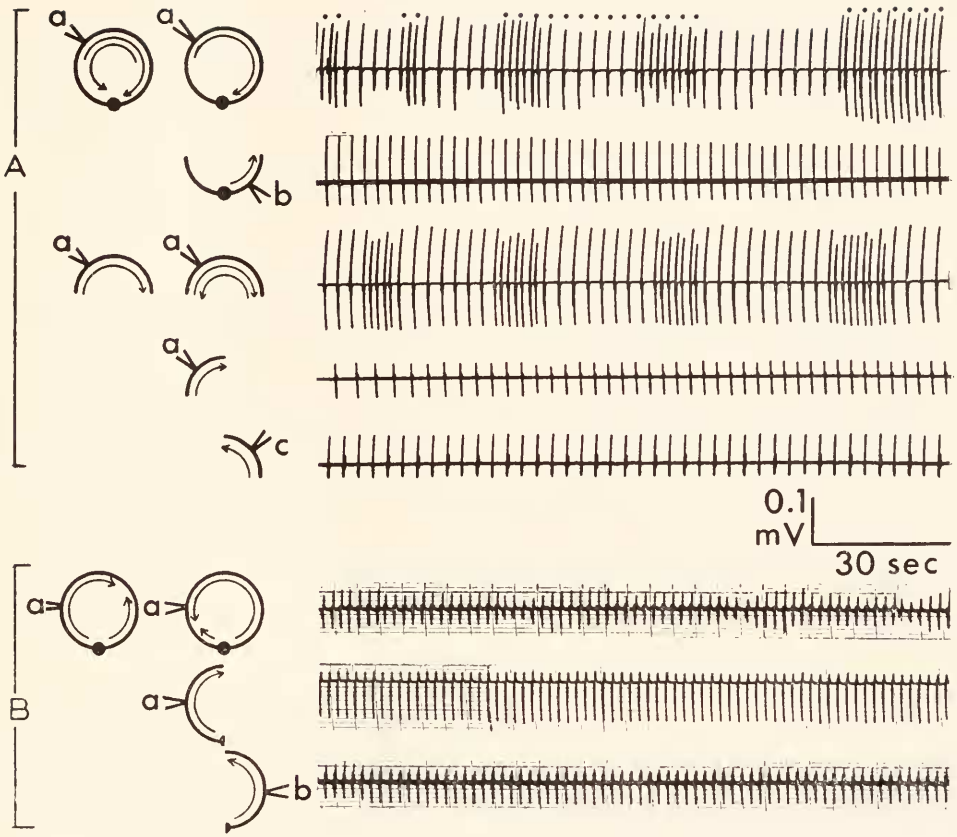

FIGURE 1. Action potentials recorded from isolated pacemaker regions. These hearts were cut in two and collapsed. To the left of each trace the pacemaker region is represented in cross-section either as a circle or, after sectioning longitudinally, as parts of a circle (the raphe is the dot). The electrode positions are alphabetized. The arrows represent directions of conduction which were determined with 3 additional electrodes (not shown). Two arrows passing each other indicate alternating directions of conduction resulting from the activity of two pacemakers (which gives rise to a doubling in beat frequency). Two arrows meeting indicate that the waves of excitation resulting from the activity of two pacemakers cancelled each other. A. Trace 1: Note the periodic doubling in beat frequency. This results from the activity of an ectopic center (action potentials indicated by dots) alternating with the primary pacemaker. The diagrams show directions of conduction during the doubling of beat frequency (left diagram) and when only one center was active. Trace 2: The heart arm has been split in two. Note that this split heart has the same beat frequency as either center in trace 1. Trace 3: Note that the pattern of the doubling in beat frequency was the same as in trace 1. Traces 4 & 5: The myocardium as shown in trace 3 was split. Note that the frequencies of these segments were about the same as that in trace 2 and the frequencies of each center in traces 1 & 3, indicating that all segments of the ring of pacemaker cells had about the same pacemaker capabilities (amplitude was altered by a change in suction). B. Note the periodic, gradual change in signal amplitude in trace 1. A change in signal amplitude resulted from a change in the direction of conduction and the activity of two pacemaker centers. The waves of contraction opposed each other as shown in the diagrams to the left of trace 1. The heart was split in two as shown in the diagrams to the left of traces 2 & 3 and the spread of excitation was unidirectional in each segment.

water was placed over the heart piece, which was pressed until flattened. Sea water (0° C.) was perfused onto one end of the slide and removed at the opposite end by aspiration. With this method of perfusion, pieces of heart tissue continued to contract for several hours. Visual observations of contractions were made with a phase contrast microscope.

Results and Discussion

A. *Patterns of pacemaker activity*

With the exception of conditions to be discussed below, only the end regions of the heart showed pacemaker properties, *i.e.*, contractions always started at one or the other end, or at both ends of the heart; they did not originate anywhere else (see Kriebel, 1968a, for details).

Collapsed hearts *in situ* or in isolation often showed a periodic doubling in beat frequency in one end of the heart. By splitting the heart wall longitudinally, it was possible to separate strips that had the slower rhythm observed in the periods between the times when "doubling" occurred (Figs. 1A and 2A).

In some hearts, generally collapsed, a second active pacemaker could be excited by locally heating a pacemaker region; *i.e.*, in addition to the general increase in frequency, the warming often resulted in a sudden doubling in the number of beats per unit time (see Kriebel, 1968a, for details). This can be readily explained as being due to an ectopic center whose beats occurred between those of the usually active pacemaker.

A second pattern of beating was detected when recording with four electrodes placed around the ostium of an intact but isolated heart, but in contrast to the above pattern of doubling, beat frequency remained the same. In these cases the signal sequence changed, indicating that the direction of conduction had changed and that two pacemakers were active, one on each side of the raphe. The change in the direction of conduction usually changed the signal amplitude (Fig. 1B). When these preparations were split longitudinally, conduction occurred only in one direction and the signal amplitudes remained constant. The alternating dominance between two pacemaker centers in one end of the heart can be compared to pacemaker competition of both ends in the intact heart (Kriebel, 1968a). These results demonstrate that the raphe functionally isolates the pacemaker regions just as it isolates the cells of the general myocardium (Kriebel, 1967a, 1967b).

Arrythmia could be abolished in isolated collapsed hearts by filling them through a cannula and in hearts *in situ* by mechanically stimulating the animal to contract, which raised the blood pressure.

By cutting or crushing parts of the cardiovascular junction containing the pacemaker, it was ascertained that a very small portion, not more than 8% of the circumference of the ostium (6 mm.), was necessary to maintain regular pacemaker activity. During recording from pacemaker regions of collapsed hearts it was often observed that two, sometimes three, sizes of action potentials appeared, each with its own regular frequency. It was particularly revealing that one or more action potentials of a certain series would drop out (Fig. 3C) as if an ectopic center had either failed to reach threshold for active responses or that conduction to the region of recording had failed (conduction is decremental and low at the ends of

the heart, which would decrease the safety margin for conduction; Kriebel, 1967a).

Sometimes the electrical records from isolated hearts showed even more complex rhythms. Careful study revealed that the complexities could be resolved into superimposed series of activities of two pacemaker centers, each showing periods of acceleration and deceleration (Figs. 2B and 3A).

B. *Properties of the middle pacemaker (the C center of v. Skramlik, 1938)*

Already mentioned previously, under normal circumstances, only the end pacemakers are active. However, when animals with exposed hearts were mechanically stimulated to contract, the blood pressure increased to such an extent that no blood was expelled and the end pacemakers either became irregular or stopped (Kriebel,

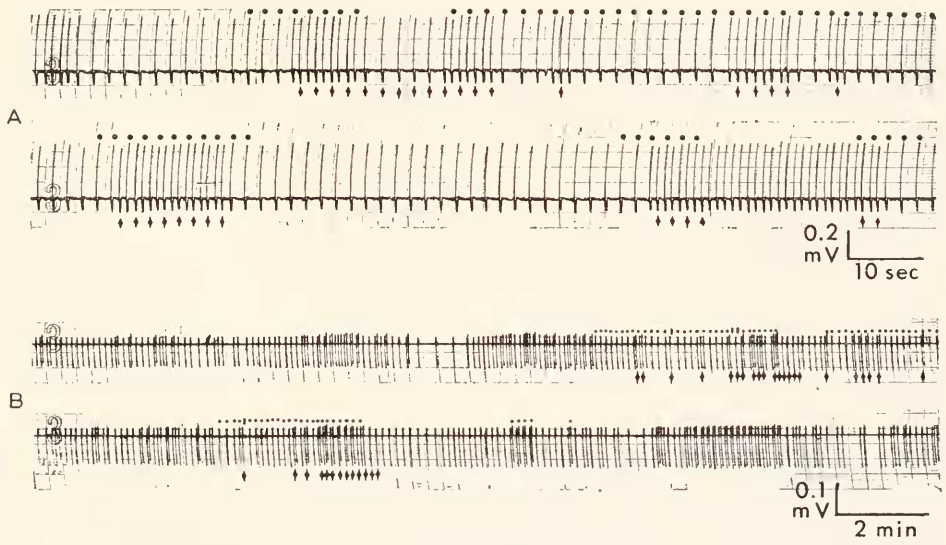

FIGURE 2. Action potentials from *Ciona* pacemaker regions. A. Traces 1 and 2 are continuous. There is little or no slowing in frequency but the frequency either doubled or decreased by half in a rhythmical pattern. Half of the action potentials recorded during the periods of doubling are in phase with those occurring during the preceding or subsequent period of low frequency beating. In trace 1, the dots indicate the action potentials that are in phase, during the doubling in beat frequency, with those preceding doubling. The diamonds indicate the phase of the remaining action potentials. Note that after the period of doubling, the action potentials were out of phase with those preceding the doubling. This activity can be explained by postulating two centers with slightly different frequencies so that a shift in phase would periodically permit both of them to drive the heart. In trace 2, one center was active all the time (dots) and the second center injected extra systoles (some action potentials are indicated by diamonds); 20° C. An opened heart arm. B. Traces 1 and 2 are continuous. In sections of this record, some action potentials in phase are indicated with diamonds or with dots and it is readily seen that the greatest frequencies result from two centers. However, the pacemakers also appear to miss beats as shown in the middle region of the first trace (*cf.* Fig. 3B). During the periods of low beat frequencies, a pacemaker accelerated and decelerated. This is shown just after the diamond symbols in the second trace (*cf.* Fig. 3A). During the periods of doubling, one center occasionally missed beats, presumably because the myocardium was refractory resulting from the prior activity of the dominant pacemaker (these misses are indicated by bars in sequence with the dots); 20° C. Heart arm containing some blood.

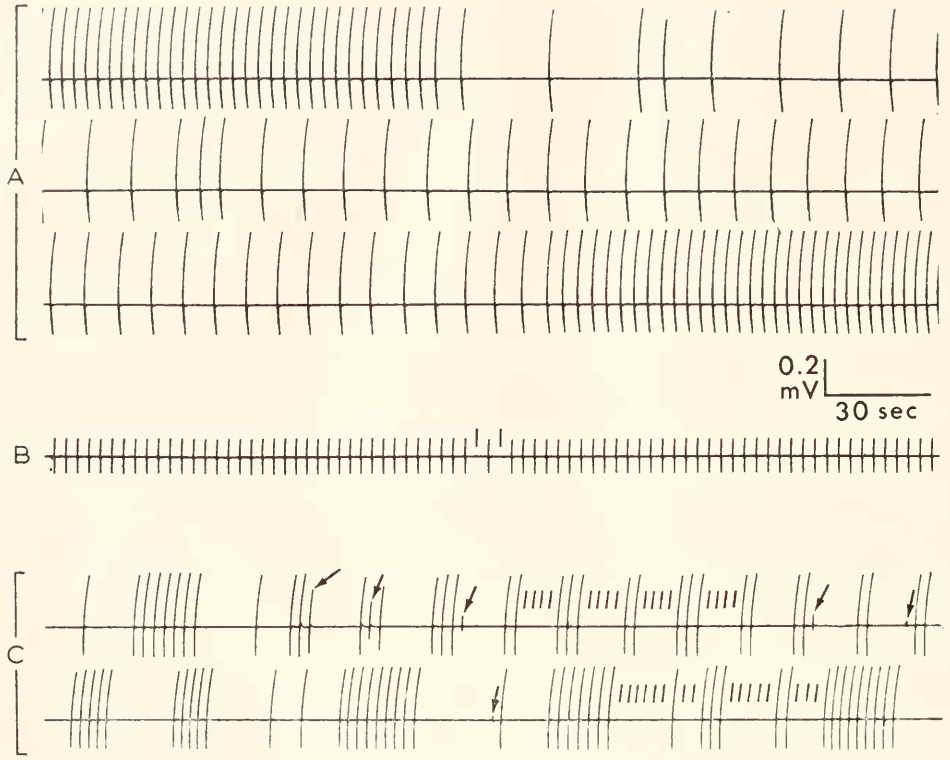

FIGURE 3. Action potentials from isolated pacemaker regions. A. These 3 records are continuous from a half-heart partially filled with blood (electrode in middle of half-heart). In the first trace, the pacemaker decreased in frequency before it stopped. In the second trace and the first half of the third trace the pacemaker gradually accelerated in a manner similar to that *in situ*. However, the beat frequency almost doubled in the middle of the third trace without a gradual increase. This probably resulted from the activity of a second pacemaker center. B. This record is from a collapsed heart arm. In the middle of the record note that two beats were missed (indicated by bars). Note that the action potentials occurring after the misses are in phase with those occurring before the misses. C. Continuous record of action potentials from a collapsed heart arm (electrode near pacemaker region). The intervals between the action potentials of the greatest amplitude are almost constant regardless of the duration of activity. The arrows point to contractions originating from ectopic centers. The bars indicate the missed beats of the pacemaker.

1968a). A few minutes after the end pacemakers had stopped, the C center became active and waves of contraction passed from the middle bend toward both ends of the heart. The frequency was always lower than that of the end pacemakers (in all four species studied).

Localized heating applied to an active C center was not as effective in increasing beat frequency as when applied to an end pacemaker. The Q_{10} of the C center in *Ciona* was found to be about 1.4 and that of the end pacemaker about 2.3 (5–25° C. range; Kriebel, 1968a). It was possible to reverse the direction of contraction by raising the temperature of the end of the heart with the inactive pacemaker or by

lowering the temperature of the end with the dominant pacemaker (Kriebel, 1968a). But when both end pacemakers were cooled to about 3° C. (so that their beat frequency dropped to only a few beats/minute) and the temperature of the middle region containing the C center was raised to about 20° C. the C center did not begin activity, although the end-to-end wave of contraction accelerated as it passed along the middle segment of the heart. Therefore a change in tension in the heart wall appears to be the only stimulus in the intact heart which will start the C center. Yet when the middle regions were isolated, the C center began to beat after a few minutes. This suggests that the change in a C center from a dormant to an active pacemaker requires that it not be driven for several minutes. The change from a dormant to an active state in the end pacemakers, after a reversal pause, requires only a few seconds (1–3 beat intervals).

The activity of the C center has been observed by many authors (Bancroft and Esterly, 1903; Hecht, 1918; Benazzi, 1935; Bacq, 1935; Sugi, *et al.*, 1965; Krijgsman, 1956).

C. *Pacemaker properties of myocardial cells*

There are many reports which demonstrate that for many species of tunicates small rings of heart tissue pulsate (see Krijgsman, 1956). However, in rings of heart it has not been determined whether the myocardial cells or the undifferentiated cells are the pacemakers. To answer this question, small pieces of tissue devoid of the raphe and the cells of the undifferentiated line were examind with a phase contrast microscope. They were observed to contract rhythmically, indicating that the cells of the general myocardium have pacemaker properties. Little or no activity was observed initially after isolating a small piece of tissue but after a few minutes 20–50% of the cells contracted at frequencies ranging from a few beats/min. to 12 beats/min. (intact *Ciona* hearts beat at about 20 beats/min.). Adjacent cells could beat independently. A few minutes later, the cells in small areas were contracting together until all the cells of the piece of myocardium contracted synchronously. The transition from contractions of small localized areas to synchronized beating of the entire piece of tissue was usually so rapid that the recruitment of additional fibers was seldom observed.

As in cultured chick heart cells (Smith and Berndt, 1964), there was no morphological difference between beating cells and quiescent cells. Synchronous beating of small groups of cells sometimes persisted for over an hour (also observed by Smith and Berndt, 1964). Occasionally single cells pulsated independently while all neighboring cells contracted together; however, after a period of time the aberrant cells usually followed their neighbors. Independent cell activity can be expected since the safety margin for conduction is very low and the excitability of the tissue is low (Kriebel, 1967a). For example, only local contractions were produced by stimulation with suction electrodes with tip openings less than 20 μ in diameter (Kriebel, 1967a). This means that several cells must be depolarizing simultaneously in order to generate a propagated wave of excitation. In the intact heart, dormant (or latent) pacemaker cells were being driven by the dominant pacemakers. This is substantiated by the fact that no intracellular pacemaker potentials were recorded from the cells of the general myocardium in the intact

heart (Kriebel, 1967a, 1967b). The transition from dormant to active states in the cells of the general myocardium requires more time than is required by the end pacemaker (after a pause during reversal) or by the C center. Since one cell cannot drive the heart, the appearance of an ectopic center depends on synchronized activity of several cells, each of which is contributing to the pacemaker current. Assuming that the pacemaker cells at the ends of the heart are functionally similar to the cells of the general myocardium, ectopic centers in the general myocardium are probably similar in size (number of cells) to those at the ends of the heart. Thus, the question arises: what factors integrate the pacemaker cells so that only one center is active in the normally functioning heart? Spread of excitation is by local current flow (Kriebel, 1967a, 1967b, 1968b). At the ends of the heart, the safety margin for conduction is very low because conduction velocity is low and decremental in nature (Kriebel, 1967c). Consequently, any factor that lowers the excitability of the tissue will lower the safety margin for conduction and in turn permit ectopic centers to develop (*cf.* Hoffman, 1965, for vertebrate hearts). Since arrhythmia was more frequent in isolated hearts, it seems reasonable to conclude that the safety margin for conduction was lower in sea water than when the hearts were in blood. However, in collapsed hearts (both *in situ* and isolated), arrhythmia was usually abolished by increasing the blood pressure. This indicates that stretch may increase the safety margin for conduction in the pacemaker region. Either one pacemaker center could drive the other potential pacemaker cells in the end pacemaker region so that they become dormant pacemakers or all of the cells in the end pacemaker region show pacemaker potentials but are driven by one center.

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Summary

1. The primary pacemakers in the tunicate heart are located near the cardiovascular junctions close to the raphe that connects the V-shaped heart to the pericardium. However, small isolated pieces of the ring of myocardial cells at the ends of the heart were found to have nearly equal pacemaker capabilities. Arrhythmia in one primary pacemaker region was found to result from activity of two or more centers.

2. Following isolation of a small piece of tissue in sea water, 20–50% of the cells were observed to pulsate and usually within a few minutes all cells contracted synchronously. Reversals in the direction of conduction in strips of myocardium without any primary pacemaker region were observed.

3. The C center located in the middle of the heart was found to be dormant during normal heart activity but in opened animals it was activated by increasing the blood pressure.

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